UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

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Stephen L. Dobson, Ph.D.
MosquitoMate, Inc.
2520 Regency Rd. Suite B
Lexington, KY 40503

Subject: Experimental Use Permit Amendment and Extension Issued for 89668-EUP-3 (Issued June 22, 2017) to Also Allow for Releases of *Wolbachia pipientis*, *ZAP* Strain in Male *Aedes albopictus* (ZAP Males®; EPA Registration Number 89668-4; Including Egg, Larval, Pupal, and Adult Life Stages) in Florida, Hawaii, Texas, and Virginia.

Experimental Use Permit No.: 89668-EUP-3
OPP Decision No.: 532788
Effective Dates: For ZAP Males®: Immediately until December 31, 2019 (pesticide applications and associated activities, e.g., collection of field data).
Quantity Authorized: 384,000,000 *Wolbachia pipientis ZAP*-Strain infected male *Aedes albopictus* mosquitoes (ZAP Males®), weighing 5,672 pounds (2572.78 kilograms) and containing 0.168 ounce (4.763 gram) of active ingredient.
Acres Involved: 26,969 (13,485 in 2018 and 13,485 in 2019)
Changes to EUP for *w*AlbB Strain in *Aedes aegypti*: None

Dear Dr. Dobson:

On the basis of the information furnished by you, the subject Experimental Use Permit (EUP) under section 5 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) is hereby amended and extended to include the pesticidal active ingredient *Wolbachia pipientis*, *ZAP* Strain in male *Aedes albopictus* (ZAP Males®; EPA Registration No. 89668-4). The EUP, which is authorized only in the states of Florida (FL), Hawaii (HI), Texas (TX), and Virginia (VA), will evaluate the active ingredient's effectiveness in suppressing and eliminating *Aedes albopictus* mosquitoes at particular sites in St. Johns County (FL), Honolulu County, (HI), Harris County (TX), and Suffolk (VA). Shipment and/or use under this permit are subject to the provisions of 40 CFR Part 172.

Prior to shipment and/or use of this material, you must consult with the pesticide regulatory officials of the states in which you will conduct your experimental program and obtain a state permit or license if such is required. Amendment and extension of this Federal permit does not negate the need for permission from individual states. Prior to initiating this experimental program in any state, you are required to notify the lead agency of the states in which you will conduct your experimental program of the specific testing program (when, where, how much, etc.). Failure to obtain that permission may result in revocation or modification of the EUP.

You must provide notice to the state pesticide regulatory agency at least 72 hours prior to the application of this experimental pesticide product. You must also provide a copy of this authorization letter and the stamped, accepted EUP labeling to all cooperators, participants, and users prior to the initial pesticide application made in accordance with this EUP. The U.S. Environmental Protection Agency (EPA) will notify the relevant EPA Regions of the approval of this EUP by sending copies of this EUP authorization letter and the stamped, accepted EUP labeling to them.

Based on the experimental program submitted, this product may be shipped for use under this EUP to the states of Florida, Hawaii, Texas, and Virginia for the next experimental period, effective immediately until December 31, 2019. The acres and amounts permitted per state are as follows:

Table 1: Approved acreage and amounts of the *Wolbachia* ZAP-strain in *Aedes albopictus* for 2018 and 2019

Location		Acres (test + control)		Number of Mosquitoes		Weight of Mosquitoes (lbs)		Active Ingredient (oz)	
State	**County**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
Florida	St. Johns	700	700	48,000,000	48,000,000	709	709	0.021	0.021
Hawaii	Honolulu	5,499	5,499	48,000,000	48,000,000	709	709	0.021	0.021
Texas	Harris	886	886	48,000,000	48,000,000	709	709	0.021	0.021
Virginia	Suffolk	6,400	6,400	48,000,000	48,000,000	709	709	0.021	0.021
Total per year		13,485	13,485	192,000,000	192,000,000	2,836	2,836	0.084	0.084
Total 24 months		**26,970**		**384,000,000**		**5,672**		**0.168**	

Further, the amendment/extension of this permit continues to allow testing and releases of *w*AlbB-Strain infected *Aedes aegypti* mosquitoes until December 31, 2018, as outlined in the Letter of Issuance on June 22, 2017. There are no changes to the existing EUP with regard to the *w*AlbB -Strain in *Aedes aegypti*:

Table 2: Previously approved of the *w*AlbB-*Strain* in *Aedes aegypti* for 2018 and 2019 (issued June 22, 2017)

Location		Acres (test + control)		Number of Mosquitoes		Weight of Mosquitoes (lbs)		Active Ingredient (oz)	
State	**County**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
California	Fresno	2,076	2,076	98,400,000	98,400,000	1,453	1,453	0.049	0.049
	Orange								
Florida	Monroe	3,554	3,554	146,400,000	146,400,000	2,162	2,162	0.072	0.072
	Lee								
	Dade								
Texas	Harris	3,200	3,200	96,000,000	96,000,000	1,418	1,418	0.047	0.047
Total per year		8,830	8,830	340,800,000	340,800,000	5,033	5,033	0.168	0.168
Total 24 months		**17,660**		**681,600,000**		**10,066**		**0.336**	

*Note: Compared to the EUP amendment/extension that was issued on June 22, 2017, the weight of the mosquitoes and the amount of active ingredient has changed in this table, based on the recalculation of the active ingredient determined to be present in the mosquitoes (w/w) and the average weight of *Aedes aegypti* mosquitoes. These recalculations do not affect the number of released *w*AlbB-Strain infected *Aedes aegypti* mosquitoes in any of the release areas for the duration of this EUP.

For the purposes of this Letter of Issuance, EPA will refer to the *Wolbachia pipientis ZAP*-Strain infected *Aedes albopictus* (ZAP Males®) and the *Wolbachia pipientis w*AlbB -Strain infected *Aedes aegypti* collectively as "*Wolbachia*-infected (male) mosquitoes" whenever both products are referenced.

You will immediately notify the EPA of any findings from the experimental uses that have a bearing on safety (i.e., the EPA requires reporting of any adverse effects from the use of or exposure to pesticides). You will also keep records of production, distribution, and performance (e.g., any knowledge of reports indicating that the *Wolbachia pipientis*, *ZAP* Strain occurring in natural populations of *Aedes albopictus* and/or the *Wolbachia pipientis*, *w*AlbB Strain occurring in natural populations of *Aedes aegypti*) and make the records available to any authorized officer or employee of the EPA upon their request.

Prior to registration or amendment under FIFRA section 3, all data requirements must be satisfied (40 CFR Part 158, Subpart V).

The labeling submitted in connection with the application for the EUP is acceptable, and a stamped, accepted copy is enclosed for your records. This labeling must be used for all shipments under this EUP and must be in possession of the user at the time of pesticide application.

You must provide a final report at the conclusion of these experiments. This final report shall include all of the items set forth in 40 CFR § 172.8(b)(2). In addition, you must perform the following testing and monitoring and include the results of this testing and monitoring and other information specified below in the final report:

(1) Monitor the mosquito population used to produce *Wolbachia*-infected male mosquitoes for possible infection with pathogenic viruses, such as dengue; Zika; Eastern equine encephalitis; vesicular stomatitis; West Nile virus; other arboviruses; and lymphatic, subcutaneous, and serous cavity filariasis.

(2) Monitor mosquitoes at the release sites to verify the absence of *ZAP*-Strain infected female *Aedes albopictus* mosquitoes and *w*AlbB -Strain infected female *Aedes aegypti* mosquitoes. If polymerase chain reaction (PCR) is used to detect the respective *Wolbachia* strains, describe how these female mosquitoes will be distinguished from the indigenous female mosquitoes that have mated with either the *w*AlbB-Strain infected *Aedes aegypti* males (in the case of *Aedes aegypti* females) or the *ZAP*-Strain infected *Aedes albopictus* males (in the case of *Aedes albopictus* females). In other words, describe how you ensure that the wild female mosquitoes do not appear *Wolbachia*-Strain positive (using PCR) after fertilization with *Wolbachia*-infected males of their own species.

(3) Sample released *Wolbachia*-infected mosquitoes to confirm the claimed rate of *ZAP* Strain- and *w*AlbB-Strain infected female mosquitoes released.

(4) Monitor population density of *Aedes albopictus* and *Aedes aegypti* mosquitoes at the treatment and control sites to determine the impact of the *Wolbachia*-infected mosquito releases and establish the level of wild population suppression of each mosquito species that can be reached by using a certain *Wolbachia*-infected male to indigenous male ratio. Report the abundance of *Aedes albopictus*, *Aedes aegypti*, and other mosquito species captured in the traps at the release sites and the control sites.

(5) Record the mortality observed after shipments arrive at each release site.

(6) Assess the quality of *Wolbachia*-infected male mosquitoes after shipping by holding a subset in a cage and report survival/fitness after 2 days(?).

(7) Mark a subset of *Wolbachia*-infected male mosquitoes to be released with dust and then monitor for their recapture (mark-release-recapture). This addresses uncertainties with the released to indigenous male mosquito ratio and released *Wolbachia*-infected male mosquito survival.

(8) Collect eggs from the field over a time interval and record egg hatch rate to verify egg hatch reduction throughout the release program.

(9) Monitor and record environmental conditions (temperature, wind speed, etc.) from a local National Oceanic and Atmospheric Administration station.

(10) Provide evidence that mosquitoes treated with tetracycline and then used to create the *Wolbachia*-infected lines have been allowed to recover for at least two generations prior to release. Provide evidence that recolonization of the gut tracts of the tetracycline-cleared mosquitoes with normal resident gut microflora occurred for at least two generations to stabilize the immune systems of the *Wolbachia*-infected mosquitoes.

The EPA requests that experiments designed to determine efficacy of the *Wolbachia*-infected mosquitoes also assure:

(1) Control and treatment sites are separated by the appropriate distance, considering the type of habitat (dwellings vs. natural habitat) and available oviposition sites.

(2) Treatment and control sites occur in the same general suburb with similar characteristics (e.g., socio economics, human density, etc.).

(3) Unbiased sampling of mosquito eggs and adult females.

The EPA acknowledges that the habitat of *Aedes aegypti* is closely associated with humans and their dwellings. Water holding containers in urbanized areas provide habitat for the development of larvae; other habitats, however, such as tree cavities, leaf axils of plants (etc.) are exploited as well (CDC 2016, [HYPERLINK "https://www.cdc.gov/dengue/entomologyecology/index.html"]).

Based on this information, the EPA suggests that:

(1) The release points also be nearby human dwellings.

Furthermore, the EPA requests that:

(1) Collections of mosquitoes be conducted in a random and unbiased manner around release points near the dwellings but also extend beyond to capture efficacy extending into natural habitats (consider typical dispersal distance).

(2) Ovitraps and BG traps be used to capture data on changes in abundance of viable offspring and adult females.

(3) The range of activity (area) of BG traps be reported if this information is attainable and percent reduction of females be measured per effective BG trap area (if possible at this point).

(4) If the range of the BG traps is unknown and cannot easily be determined, then the percent reduction in females should be reported per treatment site and associated acreage.

(5) Neighboring treatment areas should be separated by 3 times the typical dispersal distance measuring between release points.

If you have any questions regarding this permit, please contact Wiebke Striegel by phone at (703) 347-0556 or via email at striegel.wiebke@epa.gov.

Sincerely,

Richard P. Keigwin, Jr.
Acting Director
Office of Pesticide Programs

Enclosure

cc: Kimberly Bingham, EPA Region 4 (Florida)
Eugene Thilsted, EPA Region 6 (Texas)
Patti TenBrook, EPA Region 9 (California)